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(21) International Application Number: PCT/US99/08968 (22) International Filing Date: 23 April 1999 (23.04.99) (30) Priority Data: 09/065,673 23 April 1998 (23.04.98) US (71) Applicant: GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US). (72) Inventors: LOWE, David, G.; 35 Via Delizia, Hillsborough, CA 94010 (US). SCHOENFELD, Jill, R.; 236 Arundel Road, Burlingame, CA 94010 (US). (74) Agents: CONLEY, Deirdre, L. et al.; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 27 January 2000 (27.01.00)
(54) Title: QUANTITATIVE ANALYSIS OF GENE EXPRESSION (57) Abstract <p>A method is provided for determining the level of expression of a gene of interest, or panel of genes of interest, in a biological sample. The method involves preparing a standard curve for each gene of interest by assaying a dilution series prepared from a total RNA in a reverse transcriptase-5'-exonuclease PCR amplification assay. A threshold cycle (Ct) for each member of the reverse transcribed-amplified is determined and plotted versus the log of the amount of total RNA in dilution series. The plot is used to determine an RNA equivalent from which the normalized RNA equivalent for the gene of interest in the sample is determined. The assay can be used to determine the effect of a treatment of the sample on the level of expression of a gene or interest or panel of genes of interest.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>HEID C A ET AL: "REAL TIME QUANTITATIVE PCR" GENOME RESEARCH,US,COLD SPRING HARBOR LABORATORY PRESS, vol. 6, no. 10, October 1996 (1996-10), page 986-994 XP000642795 ISSN: 1088-9051 cited in the application the whole document</p> <p style="text-align: center;">--- -/--</p>	1-22



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/08968

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GIBSON U E M ET AL: "A NOVEL METHOD FOR REAL TIME QUANTITATIVE RT-PCR" GENOME RESEARCH, US, COLD SPRING HARBOR LABORATORY PRESS, vol. 6, no. 10, October 1996 (1996-10), page 995-1001 XP000642796 ISSN: 1088-9051 cited in the application the whole document	1-22
A	DUKAS K ET AL: "QUANTITATION OF CHANGES IN THE EXPRESSION OF MULTIPLE GENES BY SIMULTANEOUS POLYMERASE CHAIN REACTION" ANALYTICAL BIOCHEMISTRY, US, ACADEMIC PRESS, SAN DIEGO, CA, vol. 215, no. 1, November 1993 (1993-11), page 66-72 XP000413712 ISSN: 0003-2697 the whole document	1-22
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A	WO 97 36535 A (UNIV TEXAS) 9 October 1997 (1997-10-09) page 47-59 page 68; example 1	1-22
P, X	SCHOENFELD ET AL: "Distinct molecular phenotypes in murine cardiac muscle development" JOURNAL OF MOLECULAR AND CELLULAR CARDIOLOGY, vol. 30, no. 11, November 1998 (1998-11), pages 2269-2280, XP002123981 the whole document	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/08968

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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